

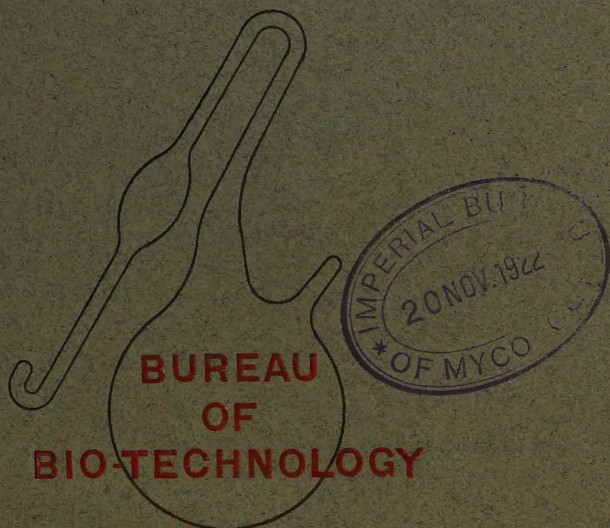
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# BULLETIN No. 7

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□ OCTOBER, 1922 □

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CONTRIBUTIONS FROM THE  
BIOLOGICAL, CHEMICAL  
AND PHYSICAL RESEARCH  
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## FOREWORD

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AT the time this issue is ready for distribution two important Exhibitions—the Brewers' Exhibition and the Imperial Fruit Show—will be in progress in London. Our departments of Fermentology and Horticulture, respectively, will be represented at these Exhibitions, and we offer our readers a very cordial invitation to visit our Stand at either or both places.

Advances in the brewing industry have been marked by, and, indeed, have been controlled by progress in Bio-technology. When Leuwenhoek, in 1680, first observed the spherical form of common yeast cells, and Persoon, some 150 years later, described those cells as fungi, neither could visualise the enormous influence their observations and discoveries would have on botany, physiological chemistry and industry—particularly the brewing industry. What is to be seen at the Brewers' Exhibition is mainly the outcome of an intensive study of the yeast cell and the application of the knowledge derived from it.

Berkeley, Worthington G. Smith, and other mycologists in their early studies of plant diseases could hardly have suspected that within a century of their first investigations there would have developed the science of Phytopathology—a branch of Bio-technology. Yet, to-day, there is a vast organised service for the study and control of fungus diseases and insect pests, and more and better fruit represent some of the many benefits that have resulted from its activities.

MURPHY & SON, LTD.

*October, 1922.*

# Study of the Causation of "Ropiness" in Worts and Beers

## II.

**B**EFORE proceeding further we may consider ropiness as it occurs in certain materials other than beer. Ropiness frequently occurs in bread, and several investigations of this phenomenon are on record. The names of Pflügge, Laurent, Watkins and others were associated with preliminary studies of the causal agent of "ropy" bread, and in 1917 their work was supplemented by a report of the (War) Food Committee of the Royal Society.

The causal organism in this instance has been shown to belong to the *B. mesentericus* group of bacteria, and the ropiness is apparently the result of the joint action of two or more extracellular enzymes acting upon the material. The slimy substance in this case is most probably of the nature of a mucin, and its formation is attended by a decomposition of a putrefactive rather than a fermentative character. *B. mesentericus* really signifies a group of very similar bacilli and not a particular species. The organism is a moderately long, Gram positive bacillus which forms endospores.

Although the source of infection in occurrences of ropy bread may usually be assumed to have been the grain, upon which large numbers of bacilli of the mesentericus group are generally present, it is very improbable that ropiness of brewery liquors could be due to this cause. Organisms of the *B. mesentericus* type are actively proteolytic; they are quite sensitive to acidity variations

and their optimum reaction is probably in the neighbourhood of pH 7 (neutrality). The reaction of different beers usually lies between pH 5 and pH 4, so that, even in the case of a heavy infection of a beer by an organism like *B. mesentericus*, it could hardly be expected to flourish under such an unfavourable condition. Nevertheless, since this type of organism is so universally distributed, infection may conceivably occur in the brewery at a time when conditions are more favourable to its growth, and detrimental effects will then result. However, in such a case a definite decomposition of the materials in solution without any great increase of viscosity would be expected to result. No authentic case of ropy beer due to *B. mesentericus* has come to the writer's notice, and the rarity with which bacteria of the mesentericus type are met with in fermented liquors is noteworthy.

Ropiness and sliminess are defects not uncommon in milk. Buchanan<sup>1</sup> discusses at some length the formation of the mucilage and the organisms associated with its production. He states that ropiness or sliminess in milk may be due in certain cases to members of the mesentericus group (mentioned above), the butyric acid group, the staphylococcus group or the *Bacillus pyocyaneus* group. But in these cases an active decomposition of the substratum occurs, which is a feature uncommon in the usual cases of ropy milk. More frequently the organism concerned in the production of ropy milk are zymogenic in character, that is to say, although they obtain their nitrogen from protein substances and consequently may possess certain proteolytic properties, they generally derive their energy from the decomposition of carbohydrates. The mucilage in this case is of the nature of a gum.

Discussing the mechanism of mucilage production, Buchanan comments upon the theories that have been advanced by way of explanation.

Firstly, the contention that the gummy material is formed by extra-cellular enzymes acting upon the constituents of the milk is described as being up to the present unproven.



Secondly, the somewhat prevalent theory that the mucins or gums may be excretory products of the cells is described as an improbable explanation on the grounds that these substances are practically indiffusible.

Thirdly, we have the contention that the mucilage results from the partial solution of the bacterial capsule or cell wall. In Buchanan's opinion this is the most likely explanation, and in one sense it may be regarded as an application of the previous theory. He further states that one may observe capsules in various stages of dissolution in the ropy milk.

Fourthly, there is the possibility of large numbers or chains of organisms mechanically binding the medium together.

Fifthly, the phenomenon may be due to the associative action of two or more different organisms.

Buchanan sums up his discussion of this point with the paragraph: "It seems apparent, therefore, that slimy and ropy milk may be due either to the development of slime and gum, as a result of a more or less complete solution of capsules and outer cell walls, or to the unusual proliferation of bacteria, or to the presence of long, tangled chains. Furthermore, the slimy condition may be due to the associated action of two or more organisms."

The observations of different workers upon ropiness in beers assume a greater significance if we keep in mind the possible ways by which the mucilage may be formed. We may utilise Buchanan's summing up of the possibilities since it is pertinent to our present study.

The last two probabilities may be disposed of since microscopical examination in this instance has shown the individual bacteria to be more or less widely separated, the numbers of bacteria are not prolific, and the cells do not occur in chains. Again, since ropiness is produced by pure cultures of *Acetobacter R.*, we can eliminate the possibility of associated action.

A popular impression is that the mucilage in beers, etc., is formed by enzymes acting upon the compound in the fluid medium. Van Laer<sup>2</sup> inferred the existence of an enzyme "Viscase," but

stated that he was unable to demonstrate its existence. I am unable to obtain proof of such extra-cellular enzyme action from any other source of information. It seems most probable that ropiness in bread may be ascribed to such a cause. But in that case there is evidently bacterial activity of a different nature proceeding in a solid substratum. It would appear that one of the enzymes concerned in extra-cellular formation of mucilage in beers would necessarily be a "coagulase," since a complex synthesis occurring exteriorly to the cells in a solution would not be in accord with our general conception of enzyme action.

Unfortunately the physical character of a ropy fluid precludes the method of filtration from use in separating enzyme from bacteria. Violent shaking with toluol is necessary in order to ensure uniform mixing, and then the ropy character is mechanically dispelled. Only one experiment was performed to ascertain if this presumably bacteria-free though enzyme-containing material could produce ropiness in a sound beer. The toluol was removed under reduced pressure and the fluid used as inoculant. Although ropiness did not result after incubation, one can hardly accept the result of such a single experiment as conclusive; it is, however, suggestive in view of the remarks which follow.

The suggestion of a mucilage as a cell excretion product is untenable, as Buchanan states, on account of the fact that the mucilage is non-diffusible. It is not uncommon to hear bacterial phenomena explained upon the assumption that substances known to be ordinarily sparsely diffusible, are excretory products of the cell. This, in spite of the fact that we regard the function of hydrolytic enzymes as one of converting complex, non-diffusible compounds into simple, diffusible ones, in order that they may be assimilated by the bacterial cell.

Finally, the theory that the mucilage results from a partial solution of the cell wall or capsule seems to fit the facts of our case. We are concerned with an organism capable of forming zoogloëæ, and we are safe in assuming that zoogloëa formation is really an elaboration of the substance of the outer cell wall.



Whether the bacterial capsules or zoogloæ are simply swellings of the outer cell wall or a particular secreted substance is undecided.

Many different methods of capsule staining have been applied to *Acetobacter R.* grown under different conditions without showing that a definite capsule exists. However, by employing Muir's method with young growths upon solid media in air or old cultures grown in the absence of air, we are able to demonstrate clearly the formation of zoogloæ. In a beer made ropy by the use of a pure culture, staining methods only show very occasional zoogloæ formations. May we assume from this that the zoogloæal substance has swollen to such an extent that it has become a mucilage responsible for the peculiar character of a beer that is ropy, and, as such, is difficultly demonstrable by staining methods? Several observations seem to indicate that the supposition is justified, and, furthermore, there is good reason to believe that the physical state (semi-solid or liquid) of the mucilage depends upon certain influences of a physico-chemical character. We have to bear in mind that we are dealing with a substance in solution in colloid state, and that the actual structure of jellies is still a subject of enquiry about which little definite knowledge exists.

The term "ropiness" has a somewhat indefinite meaning: it may signify the property of pouring as a highly viscous fluid pours—oily, syrupy, gummy. That is, it expresses a certain degree of viscosity, and any solution sufficiently viscid to pour sluggishly might be described as "ropy," as, for example, a strong sugar solution. "High viscosity" might well supplant "ropiness" in a description of this character. The production of high viscosity in fluid culture media is a common property of many species of bacteria of widely different types (particularly some species of the *Cocci*), but one has not hitherto referred to the fluid as being "ropy."

However, if some mucilaginous material be formed in the beer, imparting a gelatinous character to the fluid suggestive of solidity, we have more than a state of increased viscosity. An oil or strong sugar solution may be shaken without greatly increasing

its slow rate of flow (ropiness), but the gelatinous character just alluded to is quickly dispelled by so doing. In this case we do not adequately describe the state by using the term "high viscosity."

Apparently ropiness may be either of these two types. We have been investigating an instance wherein mucilage is formed, and we use the term "ropiness" to denote the resultant state of the beer.

If we may regard this latter state as being of the nature of jellification then we may account for the sudden great increase of viscosity occurring at a slight increase in concentration of the mucilage. On the other hand, to increase the viscosity of a sugar solution correspondingly an increase in concentration would be necessary which would be beyond the range of probability in microbic activity. Therefore, in cases of increased viscosity without the production of some jellifiable substance one would not expect the "ropiness" to be nearly so marked as if jellification had occurred.

In either case the affected beer is unsaleable, and the distinction may seem an unnecessarily fine one from the practical standpoint. Nevertheless, in scientific enquiry the point is important because we have instances of ropiness having been produced by different organisms, and several observations on the conditions or materials influencing "ropiness" are not in accordance with one another. The question arises as to the exact meaning of the word "ropiness" in the records of past investigations. It is conceivable that the use of more exact terms might have resulted in two or more chains of evidence referring to different types of ropiness, in place of the somewhat heterogeneous mass of information now in our hands.

Our suggestion is that the cells of *Acetobacter R.* (and similar species) form considerable quantities of mucilaginous material, as is characteristic of zooglæa-forming bacteria, and that this mucilage swells and partly goes into solution in the beer. The physical character of this mucilage may then be affected by such



a consideration as concentration of hydrogen ions, and we might expect that the stability of the state of jellification would be influenced by an increased hydrogen-ion concentration.

As an illustration of the effect of altered hydrogen-ion concentration upon a carbohydrate jelly, the difficulty of preparing a solid agar medium with an acidity beyond the region of pH 4 may be quoted. All bacteriologists are familiar with the difficulty of preserving the solidity of their agar jelly in slightly acid media. This of course is most apparent in the preparation of beer agar where the H.I.C. of the beer is approximately that at which the agar jelly becomes unstable.

Can we explain the occurrence and disappearance of the character of ropiness upon these grounds?

Ropy beers are not as a rule unduly acid; many samples of ropy beer retained for long periods by excluding air have not acquired an acidity greater than pH 4.4 to pH 4.0; ropiness disappears when acid is developed. The stability of all jellies is affected by shaking, and shaking dispels ropiness.

When a large amount of alcohol is added to a ropy beer the mucilage can be seen to shrink under the dehydrating action and ultimately to coalesce to form a glutinous mass. This behaviour is characteristic of recognised jellies under similar conditions. This same glutinous mass is obtained by adding alcohol to beers from which ropiness has disappeared on account of acid production. This would appear to discount the theory that there is an actual combination between the mucilage and the acid, and to indicate that the change is merely physical.

The mucilage substance when boiled with glacial acetic acid is not dissolved but becomes very brittle, and when the brittle material is returned to water it behaves precisely as it did before treatment with the acid. The addition of dilute acid to a solution of the mucilage substance does not produce a precipitate. One method of demonstrating bacterial capsules is to "fix" the capsules with glacial acetic acid before staining them.

Van Laer stated that the addition of acid to beer prevented the occurrence of ropiness and that it also removed the ropiness when once it had been formed. Lindner also found that acidity retarded the production of ropiness by his *Pediococcus cerevisæ*. Our results corroborate this, as the following table will show.

Equal quantities of beer were inoculated and incubated under reduced oxygen tension.

|                             | Ropiness<br>after 7 days. | Stability of Ropiness<br>after 4 weeks. |
|-----------------------------|---------------------------|-----------------------------------------|
| Beer + calcium carbonate    | very ropy                 | remaining ropy                          |
| Beer alone ... ..           | very ropy                 | losing ropy character                   |
| Beer + alcohol (5%) ...     | ropy                      | no longer ropy                          |
| Beer + acetic acid (1%) ... | slightly ropy             | mobile                                  |
| Beer + lactic acid (1%) ... | not ropy                  | mobile                                  |

Van Laer explained this on the assumption that the "coagulated" carbohydrate was dissolved by the acid. But in our case it is found that the purified mucilage substance is not more soluble in dilute acetic acid than it is in water, neither is there any appreciable difference in the degree of swelling. Moreover, it has been noted that in order to ensure uniform mixing of acid added to a ropy beer violent shaking is necessary which, in itself, will disperse the ropy character. Experiments with dye solutions have shown that four or five days may elapse before 1 cc. of dye will diffuse throughout 20 ccs. of a ropy beer. So that it is difficult to observe the exact effect of adding acid to a ropy beer.

It has been pointed out previously that the amount of oxygen available is a decident of mucilage formation, and the following laboratory data will demonstrate this fact.



|                                                               | ACIDITY (after 3 weeks)                                     |                                  | Ropiness.            | Remarks.                                              |
|---------------------------------------------------------------|-------------------------------------------------------------|----------------------------------|----------------------|-------------------------------------------------------|
|                                                               | cc. $\frac{N}{2}$ NaOH reqd. for 25 cc. liquor (Methyl red) | Indication of Brom. phenol blue. |                      |                                                       |
| Racking sample, in full stoppered bottle                      | 1.6 cc.                                                     | purple                           | ropy                 | Slight turbidity ; no film                            |
| Ditto, large surface exposed in atmosphere of CO <sub>2</sub> | 1.7 cc.                                                     | purple                           | ropy                 | Slight turbidity ; no film                            |
| Ditto, small surface exposed in air                           | 13 cc.                                                      | green                            | less ropy            | Turbid ; film ; granular deposit of broken film       |
| Ditto, larger surface exposed in air                          | 21 cc.                                                      | yellowish green                  | viscous but not ropy | Turbid ; film ; heavy granular deposit of broken film |

From the above it is obvious that the degree of ropiness varies inversely as the acidity which, together with turbidity and film production, is influenced by the amount of oxygen available.

Corresponding observations may be made on the growth of *Acetobacter R.* upon a solid medium (yeast or wort agar). When this bacterium is grown upon solid media in air it first appears as a hyaline, gelatinous mass which, after a few days, becomes cloudy and more or less liquid. Growth in absence of air results in a hyaline, sticky, gelatinous mass which retains these characteristics indefinitely. The use of indicators shows that in the first instance the acidity is considerably greater than it is in the latter instance.

The dextrans and kindred compounds have considerable tendency to polymerise, and it is my contention that the turbidity of the beer, and also the cloudiness developed by the growth of

the organism upon solid media, is due to polymerisation (union of molecules) of the mucilaginous compound.

Partially purified mucilage substance was prepared by cultivating *Acetobacter R.* in beer until ropiness was very marked (after five to seven days) and then adding excess of acetone or alcohol. The mass separating out was then rinsed and shaken up with distilled water. The mucilage was then again separated and the same treatment repeated. Dehydration by alcohol followed and then the hard material so obtained was ground up and heated at 100°C. for several hours. It was found particularly difficult to remove the last traces of alcohol or acetone.

This material swells in water and after shaking is taken into solution showing a marked Tyndall effect. The addition of alcohol produces a "gel" type of precipitate.

The amount of nitrogen estimated by Kjeldahl's method was found to be 1.018 per cent., calculated on dry mucilage. Such a small quantity of nitrogen may surely be accounted for by the presence of bacterial proteins and other nitrogenous impurities from the beer. The substance gives a slightly, almost imperceptibly positive xanthoprotein reaction. Other protein reactions are negative.

Hydrolysis by mineral acid has yielded somewhat variable results. In one experiment the amount of reducing sugars estimated by Fehling's solution was 89.5 per cent., calculated as glucose from dried powder. Polarimeter readings were not in accordance with this figure. Qualitative tests for levulose and pentoses did not give positive results. In this experiment there was 0.63 per cent. of insoluble matter (on dry powder) remaining after hydrolysis.

No discernible amount of ash was obtained. Van Laer stated that he obtained an acetone precipitate of high ash content. In our employment of acetone and alcohol we have ignored a granular precipitate that was occasionally formed because we have obtained it from sound beers.

The usual tests applied to the material all lead to the conclusion that the substance is of the nature of a dextran. The colouration by iodine and sulphuric acid is reddish-brown.

*Acetobacter R.* has not produced ropiness in the absence of beer or wort. The results of a series of experiments carried out with the object of discovering whether any particular material used in brewing was a deciding factor are shown in the table which follows.

Horizontally the different materials used in the experiment are denoted. Vertically the same materials are represented as having been added in small amounts to each of the experimental infusions. It will be seen that each infusion has been employed alone and also in conjunction with small amounts of each of the materials. Each infusion was inoculated with *Acetobacter R.* and incubated. The occurrence of marked ropiness is signified "+," slight ropiness "+," no ropiness "O."

| In<br>presence<br>of :— |     |     |     | Yeast | Hops | Wort | Beer, bitter | Beer, heavy | Beer, light | Glucose | Malt |
|-------------------------|-----|-----|-----|-------|------|------|--------------|-------------|-------------|---------|------|
| Malt infusion           | ... | ... | ... | O     | O    | +    | O            | +           | +           | O       | O    |
| Glucose, commercial     | ... | ... | ... | O     | O    | +    | O            | +           | +           | O       | O    |
| Beer, light             | ... | ... | ... | +     | +    | +    | +            | +           | +           | +       | +    |
| Beer, heavy             | ... | ... | ... | +     | +    | +    | +            | +           | +           | +       | +    |
| Beer, bitter            | ... | ... | ... | O     | O    | +    | O            | O           | O           | +       | O    |
| Wort                    | ... | ... | ... | +     | +    | +    | +            | +           | +           | +       | +    |
| Hops infusion           | ... | ... | ... | O     | O    | O    | O            | O           | O           | O       | O    |
| Yeast autolysate        | ... | ... | ... | O     | O    | O    | O            | O           | O           | O       | O    |



The only outstanding feature of the above is that the bitter beer required the addition of glucose before ropiness was produced, and that slight ropiness only occurred in glucose or malt when wort or beer had been added to them. It seems that glucose is essential; also some compound or condition introduced by the wort or beer, but not provided by any one material used in the preparation of the wort or beer. The nature of the nitrogenous compound available as nutriment to the bacterium is probably an important factor in the formation of the mucilage.

Up to the present experiments with different sources of nitrogen have not thrown any light on the problem. *Acetobacter R.* has been cultivated in glucose solutions each containing the following nitrogenous compounds:—peptone, asparagine, glycocoll, ammonium nitrate, and milk powder. Incubation under aerobic and anaerobic conditions produced growth without ropiness.

On several occasions whilst examining racking samples in connection with other investigations we have found bacteria typical of *Acetobacter R.*, although ropiness has not developed. In one instance glucose was added to the racking sample before incubation, and then a distinct ropiness was produced. A control without glucose remained normal. One would expect from this that low attenuation beers would be more prone to ropiness than high attenuation beers. Lindner stated that the reverse was the case in respect of his *pediococcus* which causes ropiness.

The influence of what has been termed the "accessory food factor" should be borne in mind. Leichtentritt<sup>3</sup> showed that malt, lemon and carrot extracts stimulated the growth of certain bacteria. Other evidence of bacterial requirements of accessory food substances is available. The writer has experience of the stimulating effect of malt upon several species of *Lactobacillus*, even in the presence of adequate amounts of fermentable sugars and utilizable nitrogenous compounds.

With regard to the fact that *Acetobacter R.* does not produce ropiness in milk, it is significant to note that upon milk agar, and

upon beef agar, the organism does not produce its characteristically voluminous viscid growth.

Baker<sup>4</sup> and colleagues in their investigation showed that hops had no influence upon the occurrence of ropiness; we obtain identical results with *Acetobacter R.*

A strong infusion of hops was prepared with boiling water, and varying amounts of this were added to 200 cc. portions of a light racking sample. The volume in each case being adjusted to 240 cc. with water. The liquids were then sterilised in the autoclave and when cool equal amounts of an emulsion of *Acetobacter R.* were added to each. The bottles which were all full to the narrow neck were incubated at 27°C. The results were as follows:—

| cc.<br>Beer | cc.<br>Hops<br>soln. | cc.<br>Water | Ropiness.     |                | cc. $\frac{N}{2}$ NaOH reqd.<br>for 25 cc.<br>to methyl red. |
|-------------|----------------------|--------------|---------------|----------------|--------------------------------------------------------------|
|             |                      |              | After 7 days. | After 14 days. |                                                              |
| 240         | 0                    | 0            | ropy          |                | 1.1                                                          |
| 200         | 0                    | 40           | ropy          |                | .5                                                           |
| 200         | 5                    | 35           | ropy          | all            | .3                                                           |
| 200         | 10                   | 30           | ropy          | very           | .3                                                           |
| 200         | 20                   | 20           | more ropy     | ropy           | .2                                                           |
| 200         | 30                   | 10           | very ropy     |                | .4                                                           |
| 200         | 40                   | 0            | very ropy     |                | .4                                                           |

The hops do not appear to have exerted any inhibitive action: on the contrary, the samples containing most hops developed ropiness most quickly in this experiment. The reduction of acidity by the hops is really in favour of ropiness.

The gravity of the beers used in the experimental production of ropiness has shown no influence upon the phenomenon. Stout is rendered ropy and yet remains almost brilliant after fourteen days' incubation. The behaviour of a bitter beer has already been referred to above. Two samples of this bitter beer were incubated after inoculation with *Acetobacter R.*, No. 1 sample having

received the addition of glucose. On the sixth day ropiness was very marked in No. 1, but absent from No. 2. The acidity had not changed in either case; and *Acetobacter R.* was recovered from both. Glucose was then added to No. 2, which developed ropiness after a further incubation period of seven days.

Ropiness is dispelled by shaking or by repeatedly pouring from one vessel to another, after which the fluid remains rather more viscous than it is normally. It is difficult to produce and maintain ropiness in a beer in conjunction with highly developed condition. This, in the face of other evidence, seems largely due to the agitation caused by the escape of gas under pressure when the container is opened. It has been noted frequently that beers showing a violent condition have quickly lost their character of ropiness. *Acetobacter R.* does not produce gas except when it oxidises acetic acid under aerobic conditions to carbon dioxide and water. No effect upon the condition of beers has been observed beyond the retention of the gas by the intensely viscous fluid.

A further decisive consideration in the occurrence or non-occurrence of ropiness along with secondary fermentation is the influence which other organisms, both bacteria and yeasts, may have upon the growth of *Acetobacter R.* It has been stated previously that acidity retards ropiness, so that the growth of acid-producing organisms may be expected to help in this respect, although, of course, the production of acidity is no less an evil. A sterile beer was inoculated with *Acetobacter R.* together with a mixed culture of several species of *Lactobacillus* (lactic acid bacteria). After incubation both types of organisms were growing vigorously and ropiness was intense. The addition of lactic acid retards the production of ropiness. The writer's experience of the slight antiseptic power which lactic acid undoubtedly has upon certain bacteria is that the organisms may easily accommodate themselves to its presence.

Six different yeasts grown in wort in conjunction with *Acetobacter R.* did not appear to influence ropiness in any way.



In some instances beers which developed much condition required heavy inoculation with *Acetobacter R.* before this organism could gain predominance and produce the mucilage.

Of course, all the considerations governing the predominance of a particular group of bacteria at any time must be taken into account in this case. So little systematic knowledge of the bacterial flora of beers is available ; and until we have gained a much clearer conception of the activity of the different bacterial groups we cannot hope to understand fully the part played by any one species. The cocci, species of which have been shown to cause ropiness, require to be studied much more thoroughly with a view to ascertaining their relationship to the normal beer flora. Cocci are rarely absent from beers, yet we know little of their physiological behaviour. They are very difficult to isolate by the ordinary cultural methods.

Only a knowledge of the normal flora of beers can enable us to say whether any particular case of deterioration is due to the predominance of an organism normally present or to a chance infection from external sources. This question is vital to efficient control in cases of abnormal fermentation or deterioration of beers.

The similarity between *Acetobacter R.* and the *Bacterium aceti viscosum*, described by Baker, Day and Hulton, has already been indicated ; they are doubtless very closely allied, if not identical species.

Further observations of the physiological behaviour of *Acetobacter R.* show that galactose is very slowly fermented ; glycerol is unchanged ; under anaerobic conditions glucose remains unchanged ; and good growth is obtained in Beijerinck's fluid.

If further evidence were necessary that we are concerned with one of the "acetic acid bacteria" we have it in the work of Janke<sup>5</sup>, who, in reviewing this group of organisms, states that he has isolated the *Bact. aceti viscosum* type. He has placed this organism in the "Hansenianum" group of his classification of the *Acetobacter*.

Species of *Acetobacter* may certainly be regarded as members of the normal beer flora, and whilst the usual precautions of excluding air from the barrel or bottle prevent the formation of acidity it produces a condition favourable to the production of ropiness by any mucilage-forming varieties that may be present. If there be no other outcome to our investigation than an appreciation of this fact a useful purpose will have been served.

The general impression one has gathered from the study is that bacteria of the *Acetobacter* R. type are frequently present in most beers and that exposure to air always results in the formation of acidity, whilst curtailment of the amount of air available, retarding acid formation, enables them to produce their mucilage whenever their nutritional and metabolic requirements are fulfilled.

P. HAMPSHIRE.

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# The Biological Aspects of A Defective Drainage System in a Brewery

**A**T a Meeting of the Institute of Brewing, held at the Queen's Hotel, Leeds, on September 28th, A. L. Jolliffe, Esq., of Bradford, read a paper entitled "Drainage Construction in the Brewery."

This, doubtless, will appear in due course in the *Journal of the Institute of Brewing*, and for further particulars readers are referred thereto.

As Messrs. Murphy & Son, Ltd. had investigated the biological conditions of the atmosphere and soil of a brewery before and during the reconstruction of a drainage system as outlined by Mr. Jolliffe, the writer, as their representative, was asked to offer some observations on the subject. The following notes contain the substance of his remarks, and they retain, for convenience, the form in which they were made.

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I willingly comply with Mr. Jolliffe's wish that I should offer some observations in connection with the biological condition of the atmosphere and soil of a brewery, in which the odour and general conditions proclaimed in no uncertain terms a serious state of contamination. There are two reasons that may be advanced for so doing. The first is that I can testify to the efficiency of the system of drainage adopted by Mr. Jolliffe with regard to its effect on the atmosphere of the brewery. To use language which the brewer will appreciate, the new atmosphere has a greatly



improved "nose," a state of things reflected in the better keeping property of the yeast and in the character of the deposits from the finished beers. The second reason is that the results of experiments carried out whilst reconstruction was in progress are so interesting and instructive that they may be worth placing on record.

When I first became acquainted with the brewery in question, its atmosphere, particularly that of the basement premises, was pervaded by an odour which, while embodying that of the ordinary cellar surrounded by earth, was sensibly different. If there is anything more difficult to describe to another than intermediate shades of colour it is the description of intermediate qualities of odour. There are some species of green moulds with which I associate a slight odour of camphor, and this was present, but as the brewery walls had been thoroughly cleansed, the beams swept, disinfected and whitewashed, there were no growths of any kind that could account for it. There was also just a whiff of what can only be described as a vinous odour, and reasons for this were also undetectable. It can be imagined, therefore, that there is no language that can adequately express such a mixture of "smells," and I will not further attempt to describe it. Nevertheless you will be interested to hear that I have secured what appears to be a permanent sample of this particular effluvium in the bottle that I will pass round. This was obtained, not from the atmosphere of the brewery, but from the soil, and it is being generated *in situ* by organisms growing in the sample of soil taken from beneath the floor of the brewery, as indicated below. It will add some interest to these proceedings if each member will note on a slip of paper the name of the substance which the odour recalls.\*

The state of affairs that could not be accounted for in the brewery itself led Mr. Jolliffe to investigate what lay beneath the brewery. After taking up the old drains, Petri dishes were exposed to the atmosphere in the vicinity of the culverts. In these

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\*The returned slips included the following descriptions: faint sweet, faintly fruity, musty, mouldy timber which has rotted in damp soil, slight sickly smell, no appreciable odour, etc.

experiments duplicate exposures were made, using two media, Autolysed Yeast agar and Wort Gelatine respectively. The reason for this procedure was, that in our laboratory, it had been found that an autolysed yeast medium forms a much better material for the development of the brewery organisms than does wort gelatine. This fact, with special reference to its use in bacterial work, was made known in the *Bulletin of the Bureau of Bio-Technology*, No. 5, March, 1922. Its value as a medium for the yeasts and yeast-like fungi has since been found and it is emphasised in the tabulated results which follow. It is quite certain that plate exposures, either in the brewery or elsewhere, never give a perfect idea of the number or kind of the organisms present in the atmosphere at the time of exposure. The single medium or even two or three media that will present suitable pabulum for the development of all the fungi has yet to be found, and so far as we can tell at present it is an impossible proposition. It is for this reason that one may read with less alarm than would otherwise be the case, Mr. Otto Overbeck's recent communication on "Air-borne Infection and Open Fermenting Vessels" (*Brewers' Journal*, 1922, p. 380). Mr. Overbeck refers to a gelatine plate on which one may obtain 100 colonies per three square inches during a one-minute exposure and then assumes that the organisms represented by these colonies are capable of doubling their numbers in three hours. He calculates that during the course of a brew the germs that would fall on such a surface in one hour would produce more than 25,000,000,000,000 organisms. Whilst I do not wish to minimise the risks incidental to air-borne infection, it may be said that things are not quite so bad as they appear from these figures. In the first place, during a long experience, I have never seen a state of infection in a brewery that would result in a gelatine plate showing anything like 100 colonies after an exposure of one minute ; and, in the second place, comparatively few of the organisms would find a fermenting wort a suitable medium for development.

The results from the air exposures with the two media mentioned are tabulated as follows:—

# RESULTS OF PETRI DISH EXPOSURES TO ATMOSPHERE IN THE VICINITY OF CULVERTS.

| DISH 10 cm. DIAMETER. |                                                                                      | EXPOSED FOR 10 MINUTES.                                               |                           |                                          |                |                    |                         |                    |
|-----------------------|--------------------------------------------------------------------------------------|-----------------------------------------------------------------------|---------------------------|------------------------------------------|----------------|--------------------|-------------------------|--------------------|
| No.                   | Situation.                                                                           | Blue moulds<br><i>Penicillium</i><br>& <i>Aspergillus</i><br>species. | Grey<br>moulds<br>Mucors. | Oidium<br>and<br>Monilia.                | Yeasts.        | Red<br>Torulae.    | Liquefying<br>bacteria. | Total<br>Colonies. |
| 1                     | <i>Outside old culvert—</i><br>Yeast agar ...<br>Wort gelatine ...                   | 25<br>16                                                              | 2<br>4                    | 3<br>Plate entirely overgrown with mucor | 3<br>—         | 1<br>—             | —<br>—                  | 34<br>20           |
| 2                     | <i>Inside culvert—</i><br>Yeast agar ...<br>Wort gelatine ...                        | 30<br>17                                                              | 3<br>2                    | 2<br>—                                   | 7<br>—         | 2<br>—             | 5<br>—                  | 49<br>19           |
| 3                     | <i>Culvert No. 2 at 2 ft.—</i><br>Yeast agar ...<br>Wort gelatine ...                | 14<br>6                                                               | —<br>3                    | 1<br>10                                  | 28<br>—        | 5<br>—             | —<br>2                  | 48<br>21           |
| 4                     | <i>At side of soil taken from<br/>ground—</i><br>Yeast agar ...<br>Wort gelatine ... | 17<br>8                                                               | —<br>2                    | —<br>2                                   | 60<br>moulds = | 23<br>Cladosporium | —<br>—                  | 100<br>10          |

Among the colonies described as blue moulds were *P. expansum* and *P. brevicaulis*. The *Aspergillus* species were represented by *A. glaucus* (*Eurotium herbariorum*) and *A. flavus*. Among the Mucoraceæ, the most frequent organism was *M. racemosus*, with only occasional colonies of *M. Mucedo*, which confirms my previous experience that, although the latter species is the one most commonly reported, *M. racemosus*, in the North, at any rate, is the most widely distributed species of Mucor. Most of the moulds, of course, when developing either on gelatine plates or on other media, emit an odour which can perhaps be best expressed as "musty," although the first mentioned species, *P. expansum*, has a somewhat fragrant odour with a slight suspicion of camphor. It will be seen that species of *Oidium* and *Monilia* were fairly frequent, and these are organisms that will develop in beer and will grow rapidly on yeast in store.

The yeast colonies included both culture and wild yeasts; among the latter were cells of *ellipsoideus*, *Pastorianus* and *apiculatus* types were present, but no attempt was made to differentiate them. Their presence in large numbers was naturally expected, as was also the red *Torulæ*, although 23 colonies of the latter on any one brewery plate is not a common occurrence. It is noteworthy that, although red colonies are so commonly obtained on wort gelatine plates, in this particular instance they would not have been recorded had we been working with wort gelatine alone.

The bacterial colonies were noted merely from the numerical point of view without regard to their specific nature. The work recently carried out in our laboratory with reference to ropy organisms enables us to detect with probable certainty a colony of the organism we are calling, provisionally, *Bacterium R.*, and colonies of this bacterium were present on all the plates.

The total counts, as will be seen by inspection were 34, 49, 48 and 100 respectively on the yeast agar plates and 20, 19, 21 and 10 respectively on the wort gelatine plates. It is only fair to point out that counts may have been reduced in plates Nos. 1 and 4 of the latter medium on account of a rapid overgrowth by colonies of *Mucor racemosus* and *Cladosporium herbarum*, for both



of which, wort gelatine provides a good nutrient substratum. The results do indicate, however, that the yeast autolysate is a more satisfactory medium for brewery organisms in general.

An investigation as to the relative numbers of similar organisms in the soil surrounding the culverts, and thrown out while reconstruction was in progress, was then carried out. The biological examination of soil is a much less simple matter than a similar examination of the air on account of the immensely greater number of organisms per volume that the soil contains. Taking advantage of the fact that different media are suitable for different classes of organisms, two media were again employed. These were (1) for the moulds and yeasts, "Cook's Medium No. II," according to the formula given by P. Brown and W. B. Halversen ("The Effect of Seasonal Conditions and Soil Treatment on Bacteria and Mould in the Soil," *Iowa Agric. Exp. Stat. Research Bull.*, No. 56, 1919). The formula for this medium is as follows:—

|                            |                            |
|----------------------------|----------------------------|
| Distilled water, 1,000 cc. | $K_2HPO_4$ ... 0.25 grams. |
| Dextrose ... 20 grams.     | $MgSO_4$ ... 0.25 "        |
| Peptone ... 10 "           | Agar ... 15.0 "            |

(2) For bacterial colonies, "Brown's albumen agar," Brown and Halversen, *loc. cit.*, prepared to the following formula:—

|                            |                          |
|----------------------------|--------------------------|
| Distilled water, 1,000 cc. | Egg albumen, 0.15 grams. |
| Dextrose ... 10 grams.     | $Fe_2(SO_4)_3$ ... trace |
| $K_2HPO_4$ ... 0.5 "       | Agar ... 15.0 grams.     |
| $MgSO_4$ ... 0.2 "         |                          |

The latter, while serving as a nutritive medium for both moulds and bacteria, is specially suitable for bacteria, and in no case was the plate overgrown with moulds as is usual with a medium best suited to their development. Preliminary tests showed that owing to the unusually large numbers of organisms present it would be necessary to submit the sample to enormous dilution before any estimable figures could be obtained, and the finely powdered soil was finally diluted with sterile water until 1 cc. of the mixture contained 0.000002 gram. of soil per cc. Such a dilution of course involves a considerable experimental error and the figures so obtained are of merely relative value.

The results of this examination are given in the following table:—

**ORGANISMS IN SOIL FROM BREWERY DRAINS**  
(NUMBERS EXPRESSED ON 1 GRAM. SOIL).

| No. | Source.                                    | Cook's No. 2 MEDIUM.                                           |                         |             | ALBUMEN MEDIUM. |
|-----|--------------------------------------------|----------------------------------------------------------------|-------------------------|-------------|-----------------|
|     |                                            | Moulds.                                                        | Culture of Wild Yeasts. | Red Torulæ. |                 |
| 1   | Old culvert ...                            | 1,000,000                                                      | 75,000,000              | 100,000,000 | 175,000,000     |
| 2   | Layer underneath brick-work of culvert ... | 500,000                                                        | 150,000,000             | 150,000,000 | 100,000,000     |
| 3   | 18 inches under culvert...                 | Sample not examined, preserved for inspection (odour of mould) |                         |             |                 |
| 4   | 2 ft. below culvert ...                    | 500,000                                                        | 200,000,000             | 25,000,000  | 125,000,000     |

The normal mould-fungus content of soils varies with the type from virgin soil to sewage sludge, and no data applicable to the type of soil under consideration is available. S. A. Waksman ("Soil Fungi and their Activities," *Soil Science*, 2, 1916) gives from 400,000 to 1,000,000 fungi per gram. of soil at a depth of 1". Brown and Halversen, as a result of their investigations, give a much lower count, ranging from 12,000 to 261,000. Taking into consideration the kind of nutriment that would be present in a brewery soil, our results appear to be more comparable with those obtained by the latter authors, 1,000,000 being the highest count. The species met with included all those mentioned in connection with the air analyses, and, in addition, *Mucor spinescens*, *M. circinelloides*, *Rhizopus nigricans* and *Trichothecium roseum*.

Large numbers of yeasts were again expected. An unexpected feature, however, was the extraordinary number of red *Torulæ* which proved to be present in each of the samples of soil examined. Species of red *Torulæ* are common on hops, and this fact may account for their presence in the brewery soil, otherwise, I am unable to bring forward any suggestion to explain their remarkable numbers.

Little need be said with regard to the bacteria. As already mentioned, their vast numbers are of merely relative interest and mean nothing except that the soil is simply packed with them. They owe their existence very largely to the presence of decomposing yeasts which provide them with a first-rate means of subsistence, and that they included, in addition to the fermentation bacteria, both putrefactive organisms able to decompose nitrogenous matter and bacteria of sewage origin is shown as the result of experiments to determine these points.

| No. of Sample. | Putrefactive Bacteria. | Bacteria of Fæcal Origin. |
|----------------|------------------------|---------------------------|
| 1              | ++                     | +                         |
| 2              | ++                     | +                         |
| 4              | +                      | +                         |

+ Small proportions. ++ Large proportions.

The condition of the soil surrounding the culverts and subjacent to the floor of the brewery, through which the brewery drainings were filtering by reason of many badly-fitting pipes of irregular diameter, amply accounts for the strange odours described. The carbohydrate material in sour beer, cask washings, etc., would provide fermentable solutions for the yeasts, and the alcohol and ethers produced by *Saccharomyces cerevisiæ* and other yeasts, would account for the vinous odour already mentioned. The probable presence of the *ellipsoideus* type of yeast, called by W. Frew (*Journ. Soc. Chem. Ind.*, 1898, 17, p. 561) *Saccharomyces fatidus* I, and the equally likely organisms of E. R. Moritz which produces substances having an odour of pineapple, would each contribute volatile products to the atmosphere.

Members of the *Acetobacter* group of bacteria, always present in sour beer, would oxidise the alcohol, yielding acetic acid accompanied by its characteristic odour.

The action of putrefactive bacteria on dead yeast cells would certainly be operative, and the odour arising from the metabolic products of these organisms would not improve the general character of the atmosphere, as may be imagined by those familiar with the odour of decomposing yeast.

These results and conclusions form a powerful argument for the insistence of a perfect drainage system in the brewery, and, as pointed out by Mr. Jolliffe, this is an aspect of brewery construction that has not received the attention it deserves.

F A. MASON.



# Potato Trials, 1921

## THE EFFECT OF SOIL TREATMENT ON YIELD

THE initial object of these experiments was to test the effect of chemical treatment of the soil in eradicating *Julus* Worm.

Previous to 1921, a variety of crops had been grown on plots under consideration, and these were badly attacked by the above-mentioned pest.

During these trials some important observations were made regarding the best variety of Potato, best suited to our particular soil, as well as on the effect of partial sterilisation of the soil as a result of the chemical treatment, and its effect on cropping.

### DETAILS OF PROCEDURE.

15.1.21. The four plots—Nos. 12, 13, 14 and 15—were spread broadcast with slaked lime at the rate of 8 cwt. to the acre and dug in.

28.2.21. PLOT 12 received a dressing at the rate of  $1\frac{1}{4}$  gallons of Steriliser No. 1, the active ingredient being a chlor-nitro derivative.

PLOT 13 was kept for control and received no further treatment apart from manurial. (The same amount as used on all the plots.)

PLOT 14 received 400 lbs. of Steriliser No. 2, spread broadcast and dug in.

PLOT 15 received a Steriliser No. 3, mixture of chlorinated tar acids, at 18 gallon per sq. yard.

- 9.4.21. Each plot received a mixture of  $3\frac{1}{2}$  cwt. Super, and 3 cwt Kainit per acre.
- 14.4.21. Seed potatoes sown, varieties, etc., shown in Table I.
- 30.5.21. Each plot top dressed with 2 cwt. per acre of Sulphate of Ammonia.
- 14.9.21. Crop lifted.

TABLE I.

Total yield per plot, irrespective of variety, showing effect of treatment.

| PLOT. | SEED SOWN. |      |    |          | CROP LIFTED. |       |      |              |
|-------|------------|------|----|----------|--------------|-------|------|--------------|
|       | Cwts.      | lbs. |    |          | Tons.        | cwts. | lbs. |              |
| 12    | ...        | 12   | 38 | per acre | ...          | 5     | 8    | 110 per acre |
| 13    | ...        | 13   | 39 | "        | ...          | 5     | 7    | 21 "         |
| 14    | ...        | 13   | 7  | "        | ...          | 5     | 15   | 106 "        |
| 15    | ...        | 13   | 86 | "        | ...          | 5     | 5    | 29 "         |

*Relative Table (based on 100 lb. Seed sown).*

| PLOT. |     |     |          |         |          |     |   |
|-------|-----|-----|----------|---------|----------|-----|---|
| 12    | ... | ... | 100 lbs. | yielded | 882 lbs. |     |   |
| 13    | ... | ... | 100      | "       | "        | 798 | " |
| 14    | ... | ... | 100      | "       | "        | 887 | " |
| 15    | ... | ... | 100      | "       | "        | 765 | " |

TABLE II.

Yield per variety per plot.

*Plot 12.*

| VARIETY           | SEED SOWN. |      |     |   | CROP. |       |      | RELATIVE. |
|-------------------|------------|------|-----|---|-------|-------|------|-----------|
|                   | Cwts.      | lbs. |     |   | Tons  | cwts. | lbs. |           |
| Arran Comrade ... | 15         | 81   | ... | 7 | 1     | 48    | ...  | 980       |
| Great Scott ...   | 15         | 102  | ... | 4 | 0     | 9     | ...  | 630       |
| Kerr's Pink ...   | 15         | 96   | ... | 8 | 4     | 28    | ...  | 1038      |
| Golden Wonder ... | 12         | 93   | ... | 2 | 18    | 75    | ...  | 454       |
| Arran Chief ...   | 15         | 4    | ... | 7 | 11    | 37    | ...  | 1020      |
| Majestic ...      | 16         | 41   | ... | 9 | 3     | 2     | ...  | 960       |

TABLE II—(continued).

## Plot 13 (Control).

| VARIETY.          | SEED SOWN. |      |     | CROP. |       |      |     | RELATIVE |
|-------------------|------------|------|-----|-------|-------|------|-----|----------|
|                   | Cwts.      | lbs. |     | Tons  | cwts. | lbs. |     |          |
| Arran Comrade ... | 14         | 32   | ... | 7     | 2     | 42   | ... | 1010     |
| Great Scott ...   | 18         | 71   | ... | 4     | 18    | 43   | ... | 533      |
| Kerr's Pink ...   | 15         | 78   | ... | 7     | 8     | 1    | ... | 960      |
| Golden Wonder ... | 16         | 31   | ... | 1     | 13    | 10   | ... | 270      |
| Arran Chief ...   | 13         | 59   | ... | 8     | 7     | 57   | ... | 1238     |
| Majestic ...      | 17         | 18   | ... | 8     | 3     | 85   | ... | 960      |

## Plot 14.

| VARIETY.          | SEED SOWN. |      |     | CROP. |       |      |     | RELATIVE. |     |
|-------------------|------------|------|-----|-------|-------|------|-----|-----------|-----|
|                   | Cwts.      | lbs. |     | Tons  | cwts. | lbs. |     |           |     |
| Arran Comrade ... | 16         | 69   | ... | 11    | 2     | 26   | ... | 1231      |     |
| Great Scott ...   | 13         | 68   | ... | 6     | 7     | 63   | ... | 932       |     |
| Kerr's Pink ...   | 16         | 15   | ... | 8     | 13    | 81   | ... | 1067      |     |
| Golden Wonder ... | 17         | 34   | ... | 2     | 8     | 102  | ... | 298       |     |
| Arran Chief ...   | 16         | 96   | ... | 8     | 13    | 3    | ... | 1009      |     |
| Majestic ...      | ...        | 15   | 71  | ...   | 10    | 7    | 101 | ...       | 760 |

## Plot 15.

| VARIETY.          | SEED SOWN. |      |     | CROP. |       |      | RELATIVE |      |     |
|-------------------|------------|------|-----|-------|-------|------|----------|------|-----|
|                   | Cwts.      | lbs. |     | Tons  | cwts. | lbs. |          |      |     |
| Arran Comrade ... | 18         | 32   | ... | 10    | 7     | 7    | ...      | 1132 |     |
| Great Scott ...   | 16         | 78   | ... | 7     | 6     | 34   | ...      | 877  |     |
| Kerr's Pink ...   | 15         | 94   | ... | 8     | 7     | 86   | ...      | 1047 |     |
| Golden Wonder ... | 16         | 84   | ... | 2     | 8     | 104  | ...      | 350  |     |
| Arran Chief ...   | 20         | 14   | ... | 5     | 13    | 49   | ...      | 572  |     |
| Majestic ...      | ...        | 16   | 96  | ...   | 4     | 14   | 10       | ...  | 560 |

TABLE III.

Yield calculated from 100 lbs. Seed sown, based on actual crops recorded in previous Table.

| VARIETY.          | TREATMENT—STERILISERS. |     |          |     |        |     |        |  |
|-------------------|------------------------|-----|----------|-----|--------|-----|--------|--|
|                   | No. 1.                 |     | Control. |     | No. 2. |     | No. 3. |  |
| Arran Comrade ... | 980                    | ... | 1010     | ... | 1231   | ... | 1132   |  |
| Great Scott ...   | 630                    | ... | 533      | ... | 932    | ... | 877    |  |
| Kerr's Pink ...   | 1038                   | ... | 960      | ... | 1067   | ... | 1047   |  |
| Golden Wonder ... | 454                    | ... | 270      | ... | 298    | ... | 350    |  |
| Arran Chief ...   | 1020                   | ... | 1238     | ... | 1009   | ... | 572    |  |
| Majestic ...      | 960                    | ... | 960      | ... | 760    | ... | 560    |  |

TABLE IV.

Average yield per root per variety per treatment.

| VARIETY.          | STERILISERS. |      |          |      |        |      |        |      |
|-------------------|--------------|------|----------|------|--------|------|--------|------|
|                   | No. 1.       |      | Control. |      | No. 2. |      | No. 3. |      |
|                   | lbs.         | ozs. | lbs.     | ozs. | lbs.   | ozs. | lbs.   | ozs. |
| Arran Comrade ... | 1            | 8    | 1        | 8½   | 2      | 1    | 2      | 2    |
| Great Scott ...   | 1            | 1    | 1        | 0½   | 1      | 5    | 1      | 8    |
| Kerr's Pink ...   | 1            | 11   | 1        | 8    | 1      | 12   | 1      | 12   |
| Golden Wonder ... | 0            | 9½   | 0        | 7    | 0      | 8    | 0      | 8    |
| Arran Chief ...   | 1            | 9    | 1        | 11   | 1      | 13   | 1      | 2    |
| Majestic ...      | 1            | 14   | 1        | 9½   | 1      | 4    | 1      | 0    |

TABLE V.

| VARIETY.          | AVERAGE WEIGHT OF SEED |     |      |      |
|-------------------|------------------------|-----|------|------|
| Arran Comrade ... | ...                    | ... | 2.46 | OZS. |
| Great Scott ...   | ...                    | ... | 2.26 | "    |
| Kerr's Pink ...   | ...                    | ... | 2.66 | "    |
| Golden Wonder ... | ...                    | ... | 2.07 | "    |
| Arran Chief ...   | ...                    | ... | 1.31 | "    |
| Majestic ...      | ...                    | ... | 2.63 | "    |



## DESCRIPTION OF SOIL.

The Soil may be described as a light sand, about four feet in depth, overlying a moist yellow sandy subsoil, in which water rapidly accumulates in any cavity excavated to a greater depth than five feet.

### *Chemical Analysis.*

### *Mechanical Analysis.*

|                      |                |                              |       |
|----------------------|----------------|------------------------------|-------|
| Moisture ... ..      | 7.22 per cent. | Fine Gravel (above 1 mm.)    | 6.88  |
| Loss on Ignition ... | 12.18 ..       | Coarse Gravel (1-0.2 mm.)    | 33.22 |
| Calcium Carbonate    | .31 ..         | Fine Sand (.2 mm.-.04 mm.)   | 20.96 |
| Nitrogen ... ..      | .44 ..         | Silt (.04 mm.-.01 mm.) ...   | 9.04  |
| *Phosphoric Acid     | .36 ..         | Fine Silt (.01 mm.-.002 mm.) | 7.7   |
| *Potash ... ..       | .032 ..        | Clay (less than .002 mm.)... | 2.02  |
|                      |                | Moisture ... ..              | 7.22  |
|                      |                | Loss on Ignition ...         | 12.18 |
|                      |                | Calcium Carbonate ...        | 0.31  |

\* Soluble in 1% Citric Acid.

## OBSERVATIONS ON TRIALS.

1. That Arran Comrade and Arran Chief appear to be the best varieties for growing on our soil (untreated), from the point of view of cropping.
2. Very little disease indeed was found on any of the crops.  
(Note—1921 was exceptionally dry and hot.)
3. Soil Steriliser No. 2 shows substantially an increase in the crops of some varieties, Steriliser No. 1 coming 2nd.
4. Plot treated with No. 1 Steriliser showed absence of wireworm and leather-jackets, whilst the Control was infested to some degree.

THEODORE PARKER AND A. W. LONG.

# The Suppression of Insect Pests and Fungoid Diseases

## I. THE FUMIGATION OF MALTHOUSES

UP to recent times, as has been pointed out by F. A. Mason (*Journ. Inst. Brew.*, 27/1921, p. 346), the weevil has been regarded as the principal pest of the malthouse, and although very aggravated cases of its presence are on record, its extermination in normal cases can be brought about by several well-known means.

But with the introduction of so much doubtful foreign barley during the War, a variety of other and more serious pests have made their appearance, and it has become an important matter to consider the whole question of malthouse pests and their ravages and to find new and improved methods for eradicating them.

The most important of these undesirable visitors is *Trogoderma khapra*: when once it has gained a footing in a malthouse it is an extremely difficult matter even to get the pest under control, let alone to completely exterminate it, and, undoubtedly, thousands of pounds per annum are lost owing to the depredations of this insect. For, astonishing as it may appear, the larvæ can exist for a considerable length of time without either food or air, and they are surprisingly resistant to most forms of chemical sprays and fumigants.

It has been estimated that during a season the damage done to a consignment of malt by the pest in a case of bad infestation approximates to something like 40 per cent. actual loss of malt.

Unfortunately, the pest seems to be spreading, and it is to be expected when one finds it so frequently in imported barley which becomes widely distributed throughout the maltings of this country.

The larvæ which are responsible for the damage are covered with hairs, which enables them to adhere to sacks, and these are amongst the main agents in the universal spread of the trouble.\* All sacks coming into the premises and of suspicious origin should be thoroughly stoved in the presence of an effective disinfectant as in the case of clothing from an infectious disease patient.

From numerous observations made, the infestations only appear where the malt bins are adjoining the kilns or kiln shafts—*i.e.*, where the temperature approximates to 90°–110° F.

Malt stored in cold bins appears to be unaffected, simply because the optimum temperature for the growth and multiplication of the pest is about 110°F. and so far it has not become acclimatised to a lower temperature, but although quiescent, its presence is of course always a source of potential danger. Generally speaking, the greatest infestation occurs round about the walls, etc., adjoining the kiln or kiln shafts, and it is quite a common sight after emptying an infested bin to find these places literally covered with a coating of larvæ, sometimes 6" in thickness.

The larvæ migrate from one part of the building to another, seeking warmer conditions, but they have not, so far, been observed on the barley floors, etc.

A point which makes the control of the pest difficult, is, that the larvæ get into deep-seated cracks and crevices; there they remain, and consequently several fumigations are necessary under pressure to reach them in their places of hibernation.

It is a common practice to annually limewash the interior walls of the malt bins and corridors, and in quite a number of cases, large blisters of limewash have been found, in which many

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\* *Bull. Bur. Bio-Tech.*, No. 2, Jan., 1921, p. 27; and No. 3, June, 1921, p. 79.

thousands of active larvæ have been present, with no visible sign of food; the only air which could reach them being that which diffuses through the film of lime and through the pores of the brick.

In one case, it was particularly noticed that the lime blisters had five consecutive coats of lime superimposed upon one another, yet underneath, a large colony of the larvæ existed.

From this observation, it was deduced that the eggs had been recently hatched or the larvæ of *T. khapra* had remained without food for five years.

It has been found from experience that fumigation is the only really effective means of checking the ravages of *T. khapra*.

This can be carried out by fumigating the premises in sections, and it involves sealing off the infected portion of the building from the remainder. Whilst this method gives satisfactory results, it is by no means the best owing to the risk of, and difficulty in, preventing the larvæ from migrating through cracks, etc., to other parts of the premises.

Undoubtedly the best method for carrying out any remedial treatment is the fumigation in one operation of the entire floor, or, better still, the whole premises.

This latter method possesses some disadvantages owing to the difficulty of removing the whole of the malt and leaving the premises vacant for two or three weeks.

It is of course best to start fumigating during the off season, when malting operations are at a standstill.

The writer wishes to strongly impress upon those who are unfortunate enough to have this pest on their premises, the vital importance of not allowing the trouble to become serious before carrying out a system of control. The following details based upon practical experience will give some idea of the present method of ridding premises of this particular pest.



A large malthouse comprising a screening floor and hop store, eight bins and two corridors surrounding two kiln shafts, was seriously infested with *T. khapra*. The extent of the infestation was so bad that owing to the pest having hibernating quarters in deep-seated cracks of long standing, it was necessary to fumigate five times.

The premises were made as air-tight as possible by sealing up windows, ventilators, doors, etc.

The kiln fires were kept going a few days prior to each fumigation and the temperature of the bins was maintained at about 90° F., the total cubical capacity being 75,470 cub. ft.

The infested bins were situated on the middle of three floors, one above the other, hence it necessitated applying the fumigant simultaneously on each floor, as the screening floor immediately above the bins and the hop store below had also become involved.

One entrance on each floor was left open to enable the rapid exit of the operator after spreading the fumigant. These were immediately sealed up and the premises remained closed for one week. At the expiration of one week, the three floors were again opened up and the vapours allowed to disperse before entering.

The fumigant which was used was "Alvesco" Grain Fumigant, a very potent, volatile liquid which, after many months of comparative trials, has shown itself to be the only effective material which would really kill the pest.

The bins were next swept down and the dead carcasses collected. It was noted that the "Alvesco" Grain Fumigant possessed a very valuable property, namely, that it caused the larvæ to vacate their places of hibernation, and, in their search for air and desire to evade the fumes, they became victims to the fumigant.

The whole process was repeated four times, as indicated above, and the total weight of carcasses removed amounted to 2½ cwt. The total volume of fumigant used was 132 gallons.

The bins had been previously partitioned with 9"×3" boards, but it was decided to remove these completely as the pest was found in great numbers in the tongued grooves of the boards. These partitions are not to be replaced, and subsequent partitions are to be built of brick or concrete and the surfaces to be "Dukeroned" as indicated below.

In cases where the infestation is comparatively slight, it has been found that one fumigation has been sufficient to eradicate the pest, providing that the subsequent insect proofing of the premises after treatment is carried out according to the following recommendations.

#### INSECT PROOFING OF PREMISES.

Once the pest has been cleared from a malthouse, it is necessary to take such measures as will prevent it establishing itself in cracks, crevices, etc., in case of any subsequent infestation.

The object then in any system of insect proofing must be to produce an impervious surface continuous throughout on floors, walls, ceilings and corridors. By so doing, any larvæ that may have escaped the fumes in deep-seated cracks are sealed up in their places of hibernation and cannot cause any further trouble.

Also any larvæ that may be re-introduced into the bins cannot find shelter and there is a much better chance of keeping the pest under proper control.

The writer has found the following treatment the most satisfactory method for making bins, etc., insect-proof as far as humanly possible, and would recommend its serious consideration by those whose premises have been or are so infested.

The floors of bins, corridors, etc., should be preferably made of asphalt, and not wood, as the floor-board joints are favourite hibernating quarters of the pest.

These cracks could of course be caulked, but the former method gives the best results.

All brickwork, ceilings, partitions and walls should be thoroughly scraped in order to remove limewash and any loose material.

Any cracks and crevices in the walls, ceilings, etc., should be thoroughly cleared and opened out, and in the case of brickwork they should be filled in with a special fluosilicated asbestos compound, which has the advantage of not cracking when exposed to variations in temperature.

Cracks in wooden partitions should be filled in with a special bitumastic filling compound, which rapidly dries and produces an elastic finish, adhering to the wood with great tenacity. All walls should be washed down with "Superol," a compound which disinfects at the same time as preparing a surface to receive the bitumastic enamel.

All surfaces should next be painted with "Dukeron" to produce the desired enamel surface which is impervious to both insects and moisture. It dries quickly and leaves a thoroughly hard surface, which will not crack when exposed to differences in temperature.

It may seem at a first glance that these precautionary measures are superfluous, but those who have had any experience in attempting to control *T. khapra* will agree that they are essentially a vital part of the treatment.

Indeed, the adoption of the insect proofing would, as a preventative measure, save many anxious moments, a considerable amount of cash, and reduce to a minimum the risk of serious damage in case of any subsequent insect trouble.

It is hoped that the preceding notes, based on actual practical experience in grain pest control, will be of service to maltsters.

In conclusion, it is desirable to add that enquiries in connection with this important subject will be welcomed: our advice and services are at the disposal of all interested.

THEODORE PARKER.

## Notes and Comments

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It is curious how the term "bug" has become a colloquialism among bacteriologists, mycologists, proto-zoologists, and entomologists. When any of these devotees to biological science refers to his pet organisms they become "bugs." Industrialists and others are beginning to use the word in the same sense. A leather manufacturer finds a "bug" destroying his leather; it turns out to be a blue mould. A brewer reports a "bug" in his beer, which produces a foreign flavour; on investigation it proves to be a wild yeast. A horticulturist complains of the attack of a "bug" on his plants, and it may be either a bacterium or a nematode worm. This reminds us of the story of a famous Professor of zoology, an entomologist, who was reputed to be able to identify many thousands of insects at sight. His students one day attempted a joke at his expense by concocting a very respectable-looking insect made up of the legs, wings, antennæ and other anatomical parts of various insects, and they asked his opinion on what they prevaricated to be a new species. The Professor looked at the insect and then at his students and said, "It is a bug, gentlemen,—humbug."

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In repetition of the invitation cordially given in our Foreword we shall be pleased to see at our Stand, either at the Brewers' Exhibition at the Agricultural Hall, or the Fruit Show at the Crystal Palace, any of our friends who are interested in "bugs," whether they desire their riddance or their propagation.

One of our Departments has adopted the slogan,

"If it's anything to do with Fermentation,  
It's something to do with Murphys of Mortlake."

It might equally well be said,

"If it is anything to do with "bugs,"  
It's something to do with the Bureau of Bio-Technology."

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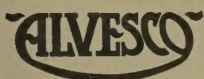
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